Nitric oxide does not contribute to the hypotension of heatstroke

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Ryan, Kathy L., Maria R. Tehrany, and James R. Jauchem. Nitric oxide does not contribute to the hypotension of heatstroke. *J Appl Physiol* 90: 961–970, 2001.—The purpose of this study was to determine whether nitric oxide (NO) contributes to the hypotensive state induced by prolonged environmental heat (EH) stress. Ketamine-anesthetized rats were instrumented for the measurement of arterial blood pressure, electrocardiogram, and temperature at four sites. Rats were exposed to EH (ambient temperature, $40 \pm 1°C$) until mean arterial blood pressure (MAP) decreased to 75 mmHg, which was arbitrarily defined as the induction of heatstroke. In addition to cardiovascular and temperature measurements, the time required to reach this MAP end point and the subsequent survival time were measured. In three separate experimental series, the competitive NO synthesis inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) was administered (0, 10, or 100 mg/kg) either before, during (30 min after initiation of EH), or immediately after EH. L-NAME administered at any of these times transiently increased MAP. L-NAME infusion either before or during EH did not alter the EH time required to decrease MAP to 75 mmHg, but L-NAME pretreatment did decrease the colonic temperature at which this MAP end point was reached. L-NAME infusion before or after EH did not affect subsequent survival time, but L-NAME administered during EH significantly decreased survival time. The administration of L-NAME at any time point, therefore, did not prove beneficial in either preventing or reversing heatstroke. Taken together, these data suggest that NO does not mediate the hypotension associated with heatstroke.

environmental heating; hyperthermia; heat stress; blood pressure

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HEATSTROKE IS A MEDICAL EMERGENCY characterized by profound hypotension, delirium, and convulsions that even now is associated with rates of mortality of 12–21% (11, 51). At this time, the physiological mechanism(s) producing the hypotension associated with heatstroke is still unknown. Adolph and Fulton (1) first suggested that, at high temperatures, peripheral vascular resistance decreases, resulting in circulatory failure. Subsequently, it was determined that hypotension precedes any fall in cardiac output, suggesting that peripheral vascular pooling of blood is the primary event (10, 23, 50). Kregel et al. (26) provided direct evidence for such a sequence of events, showing that a loss of compensatory vasoconstrictor tone to the mesenteric arterial bed and, hence, a dramatic mesenteric vasodilation precede any fall in arterial blood pressure in severely hyperthermic rats. The question then becomes, what mediates the loss of peripheral vascular resistance in the terminal stage of heatstroke? Although this is unclear at the present time, it is clear that the mesenteric vasodilation that precedes heatstroke is not due to decreases in sympathetic nerve activity or circulating catecholamines (15) or to a direct effect on the vascular contractile machinery (24).

It is now well established that pathological increases in the potent vasodilator nitric oxide (NO) occur and may contribute to the hypotensive states associated with circulatory shock models of different etiologies, including endotoxemia, septicemia, and hemorrhage (31, 45, 49). It is, therefore, plausible that release of NO could contribute to the hypotension accompanying heatstroke. Plasma nitrite/nitrate levels are elevated in heatstroke patients, suggesting that NO production has increased (2). Interestingly, Hall et al. (19) demonstrated that NO levels are not altered in arterial blood from hyperthermic rats but are elevated in blood collected from the portal vein, suggesting that enhanced release of NO within the splanchnic circulation might contribute to the mesenteric vasodilation preceding hypotension. If this were the case, blockade of NO synthesis might be expected to attenuate the subsequent hypotensive response. Such a suggestion has not yet been verified with the use of pharmacological inhibitors of NO synthesis after environmental heating (EH).

It should be noted, however, that pharmacological inhibition of NO did not reveal any contribution of NO to the hypotensive response in another hyperthermia-based model of circulatory shock induction that is accompanied by mesenteric vasodilation, namely 35-GHz microwave heating (36, 37). In this model, the shallow depth of penetration at this frequency (35 GHz) results in the deposition of energy primarily in the cutaneous

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region (14). Sustained exposure to 35-GHz radio-frequency radiation thus produces a pattern of heating that is characterized by relatively high surface temperature with only moderate heating of the body core (14). Despite the only moderate increase in core temperature, the cardiovascular responses to microwave exposure are quite similar to those accompanying larger increases in core temperature produced by EH (26). In both instances, a transient pressor response occurs during the initial phase of heating and is followed by severe hypotension as hyperthermia progresses further. Furthermore, this hypotension is accompanied by a loss of thermoregulatory compensatory vasoconstrictor tone to the mesenteric vascular bed and thus mesenteric vasodilation in both models of heatstroke induction (14, 26). Importantly, however, the onset of the hypotensive and mesenteric vasodilatory events in animals heated by 35-GHz microwave exposure occurs at a core temperature <40°C (14), whereas these events only occur in animals exposed to EH when core temperature rises >41.5°C (26). Additionally, the time course of microwave exposure (at the power level used) required for the onset of hypotension is shorter than that for hypotension induced by EH.

Because these previous results using 35-GHz microwave exposure were surprising in light of the available literature regarding NO and environmental heat stress, we questioned whether this was a result of the specific heat stress we had imposed (i.e., preferential superficial heating using radio-frequency radiation) or whether the lack of contribution of NO to the hypotensive response induced by heat stress was independent of the heating regimen employed and would also be observed in a traditional animal model of heatstroke. The objective of this study was, therefore, to determine whether NO mediates hypotension induced by sustained EH in rodents. We investigated this question using the competitive NO synthesis inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME).

MATERIALS AND METHODS

All experiments and animal care were approved by the Institutional Animal Care and Use Committee of the Air Force Research Laboratory and were conducted according to the National Institutes of Health "Guide for the Care and Use of Laboratory Animals." The animals used in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Seventy-two male Sprague-Dawley rats (Charles Rivers Laboratories), ~4 mo old and weighing between 350 and 400 g (364 ± 1 g), were used in this study. Before experimentation, animals were housed in polycarbonate cages with free access to Purina rodent chow and water. The rats were maintained on a 12:12-h light-dark cycle (lights on at 0600) in a climate-controlled room [ambient temperature (T_a) = 24.0 ± 0.5°C].

Rats were anesthetized with ketamine-HCl (Vetalar; 150 mg/kg im), with supplemental doses provided during experimentation. Ketamine administration at this dose level has been shown to produce prolonged surgical anesthesia in Sprague-Dawley rats (21) and has been used previously in our laboratory in studies assessing the possible contribution of NO to cardiovascular responses induced by thermal stress (36–38). Additionally, this anesthetic has minimal effects on temperature regulation in rats (34). In a previous report, our laboratory has more fully discussed the use of ketamine as an anesthetic (21). A Teflon catheter was placed into a carotid artery for measurement of arterial blood pressure by using a Century (model CP-01) blood pressure transducer connected to a pressure processor (model 13–4615–52, Gould). A second Teflon catheter was inserted into an external jugular vein for drug infusion. A lead II electrocardiogram was obtained by use of nylon-covered fluorocarbon leads attached by a cable to a Gould electrocardiograph/Biotach amplifier (model 20–4615–65, Gould). All measured variables were recorded continuously throughout experimentation on a Gould TA2000 recorder.

The animals were also instrumented to monitor temperature at five sites: 1) left subcutaneous (lateral, midthoracic side facing away from heat source); 2) right subcutaneous (lateral, midthoracic side facing heat source); 3) left tympanic; 4) colonic (5–6 cm postanus; T_c); and 5) tail (subcutaneous, dorsal, 1 cm from base). All temperature measurements were obtained via thermistor probes (BSD Medical) attached to a precision thermometry system (model BSD-200, BSD Medical). All temperature and cardiovascular data were analog-to-digital converted by an IBM-compatible, custom-designed physiological monitoring system with real-time graphics display and data analysis capabilities (14).

After instrumentation, animals were placed in a custom-designed EH chamber in which T_a could be raised and controlled via a thermostat. The design of the chamber was such that heated air entered the chamber through vents located in one wall. To ensure that each animal was exposed to EH in an identical fashion, the animal was always placed with its right side parallel to the vents through which heated air flowed. Airflow in the chamber was kept constant throughout both control and EH periods. While in the EH chamber, the anesthetized rat lay on a holder consisting of seven 0.5-cm (outer diameter) Plexiglas rods mounted in a semicircular pattern on 4 × 6-cm Plexiglas plates (0.5 cm thick). All instrumentation leads and catheters exited the chamber via small ports. On initiation of EH, ~10 min were required to attain the target T_a of 40°C; thereafter, T_a was controlled within ±1°C of this value. In all experiments, EH was maintained until mean arterial blood pressure (MAP) decreased to 75 mmHg. Preliminary experiments indicated that, if heating is discontinued at this point, MAP will continue to decline until death (unpublished observations). Furthermore, our laboratory has previously used this end point in other studies in which the contribution of NO to the hypotension induced by 35-GHz microwave heating was investigated (36–38). We therefore discontinued EH at this MAP end point and arbitrarily defined this as the point of heatstroke induction. After EH was discontinued, experimental parameters were continuously monitored until death ensued.

Three separate experimental series were performed. In each of these experimental series, the NO synthesis inhibitor L-NAME (10 or 100 mg/kg iv) or an identical volume of vehicle (saline) was administered to separate groups of instrumented rats (n = 8/group) either before, during, or after EH. In the pretreatment series, animals were monitored at room temperature (25.5 ± 0.3°C) for 30 min, administered a bolus of either saline or L-NAME, and then monitored for another 10 min before initiation of EH. In

the second series, EH was initiated after a 30-min control period, and L-NAME or vehicle was administered 30 min after the initiation of EH. In the final series, EH was initiated after a 30-min control period and was maintained until the MAP end point of 75 mmHg, and either L-NAME or vehicle was immediately infused thereafter. Each of the three experimental series thus included three experimental groups (0, 10, or 100 mg/kg L-NAME; $n = 8$/group) and differed only in the time point at which the drug was administered. Supplemental doses of ketamine were given at 30-min intervals throughout each experiment to maintain adequate anesthesia.

L-NAME was obtained from Sigma Chemical (St. Louis, MO) and solubilized in 0.9% NaCl immediately before administration. The volume of L-NAME or saline vehicle infused was 0.35–0.40 ml in all experiments. The 10 mg/kg dose of L-NAME was used in our laboratory's previous study (36); we also chose to use a higher dose (100 mg/kg) to increase the likelihood of more complete NO synthesis inhibition.

Data analysis. To determine differences between thermal and cardiovascular responses to EH produced by L-NAME
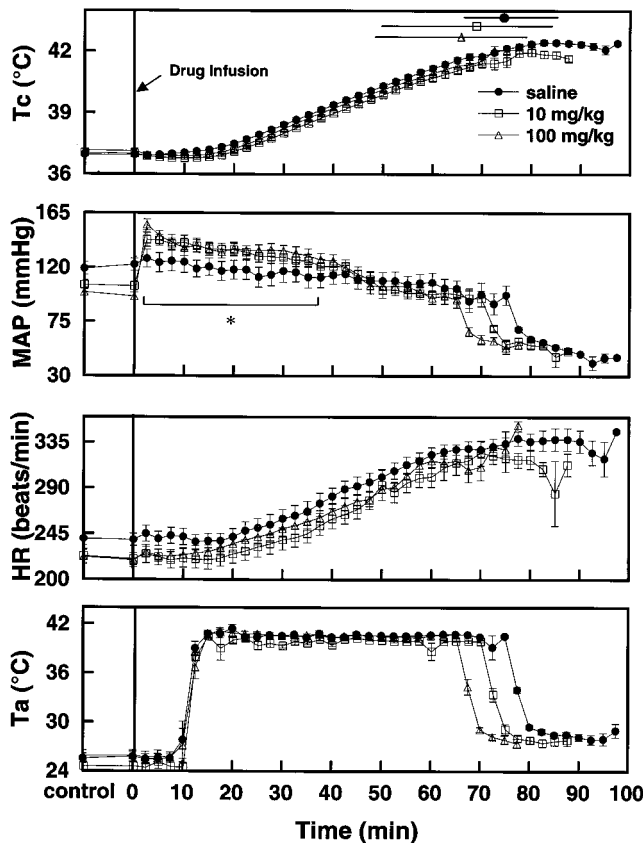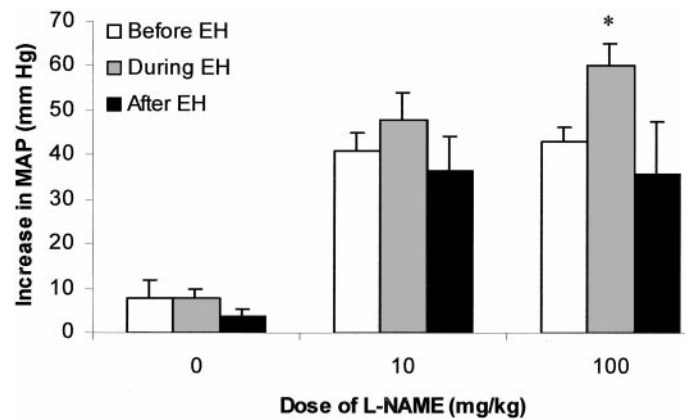

Fig. 2. Pressor responses to L-NAME (0, 10, or 100 mg/kg) administered either before EH, 30 min after the beginning of EH (during), or immediately after EH. Values are means ± SE. *Significantly different from the pretreatment value for L-NAME at the 100 mg/kg dose ($P < 0.05$).

administration, a two-way ANOVA with repeated measures was applied within each experimental series, followed by the Student-Newman-Keuls multiple-comparison test as necessary (53). A two-way ANOVA without repeated measures was used to determine differences in pressor responses to L-NAME infusion at the differing times of administration. A one-way ANOVA was used to determine differences in the heating time required to induce heatstroke (i.e., decrease MAP to 75 mmHg) and the subsequent survival time. In all of the statistical tests, results were considered significant when $P < 0.05$. All data presented in RESULTS are means ± SE.

RESULTS

Pretreatment with L-NAME resulted in significant increases in blood pressure (Fig. 1); pressor responses did not differ between the two doses (Fig. 2). Temperature measurements at all sites immediately before EH did not differ among treatment groups (Table 1). In vehicle-treated animals, the T_c at which MAP decreased to 75 mmHg was 42.2 ± 0.1°C; L-NAME treatment at either dose significantly decreased the T_c at which the MAP end point was attained. L-NAME pretreatment also attenuated the increase in tail temperature at the end of EH but did not alter tympanic or subcutaneous temperatures. Although MAP increased in L-NAME-pretreated animals at the start of EH and for the first 30 min of EH, MAP responses at later times of EH were not significantly altered by L-NAME (Fig. 1). Heart rate (HR) in all groups increased during EH; there were no significant differences in HR during EH among the three treatment groups. The EH exposure time required to attain the MAP end point was not altered by L-NAME pretreatment (Fig. 3). Additionally, the rate of change in T_c was not significantly affected by L-NAME pretreatment. Subsequent survival time tended to decrease in L-NAME-pretreated animals, although these decreases did not attain statistical significance ($F = 2.203$; $P = 0.135$).

In the second experimental series, there were no differences among groups in temperature measure-

Fig. 1. Thermal and cardiovascular responses to environmental heating (EH) after pretreatment with vehicle (saline) or N^ω-nitro-L-arginine methyl ester (L-NAME; 10 or 100 mg/kg). T_c, colonic temperature; MAP, mean arterial pressure; HR, heart rate; T_a, ambient temperature. Values are means ± SE. Solid horizontal lines in *top* show the range of times at which EH was discontinued for animals within that group, with the symbol placed at the group mean. Data points are shown only for times with 4 or more animals remaining in the group. Statistics were performed only through the values for 47.5 min because of missing data values thereafter. *Values for both L-NAME groups significantly different from value for saline group ($P < 0.05$).

Table 1. *Temperature measurements before EH and at the end of EH in rats pretreated with L-NAME (0, 10, or 100 mg/kg)*

	T_c	T_{tym}	T_{sl}	T_{sr}	T_t
Before EH					
Saline vehicle	36.9 ± 0.1	36.6 ± 0.1	35.4 ± 0.3	34.2 ± 0.3	28.2 ± 0.8
L-NAME (10 mg/kg)	37.0 ± 0.1	36.7 ± 0.1	34.5 ± 0.4	34.2 ± 0.8	26.8 ± 0.7
L-NAME (100 mg/kg)	37.2 ± 0.1	36.8 ± 0.1	35.2 ± 0.5	35.4 ± 0.4	28.0 ± 0.6
End of EH					
Saline vehicle	42.2 ± 0.1	41.8 ± 0.1	41.0 ± 0.3	39.7 ± 0.2	40.1 ± 0.1
L-NAME (10 mg/kg)	$41.4 \pm 0.3^*$	41.1 ± 0.3	39.7 ± 0.4	39.5 ± 0.6	$39.8 \pm 0.1^*$
L-NAME (100 mg/kg)	$41.4 \pm 0.3^*$	40.9 ± 0.3	40.1 ± 0.5	39.9 ± 0.4	$39.7 \pm 0.2^*$

Values are means $\pm$ SE in °C. EH, environmental heating; L-NAME, N^ω-nitro-L-arginine methyl ester; T_c, colonic temperature; T_{tym}, tympanic temperature; T_{sl}, left subcutaneous temperature; T_{sr}, right subcutaneous temperature; T_t, tail temperature. *Significantly different from value in saline-treated group ($P < 0.05$).

ments (Table 2), MAP, or HR (Fig. 4) before EH. L-NAME was administered 30 min after the initiation of EH; administration of 10 and 100 mg/kg of L-NAME during EH resulted in immediate increases in MAP that did not differ from each other (Fig. 2). The pressor response to 100 mg/kg of L-NAME was significantly higher, however, than that produced by pretreatment with 100 mg/kg. Interestingly, the time required for MAP to return to the pre-L-NAME value when L-NAME was given during EH was significantly less for both doses (23 ± 3 and 25 ± 2 min for 10 and 100 mg/kg, respectively) than the corresponding values when L-NAME was given before EH (50 ± 4 and 52 ± 5 min, respectively). Despite these pressor responses, neither the T_c at which MAP

Fig. 3. EH exposure time required to reach the arbitrary end point (i.e., MAP decreased to 75 mmHg; *top*) and the subsequent survival time (*bottom*). L-NAME was administered before EH was begun. Values are means $\pm$ SE.

decreased to 75 mmHg nor the EH time required to reach this end point (Fig. 5) differed from those in saline-treated animals. Furthermore, the rate of increase in T_c was not altered by L-NAME administration at either dose. The survival time after EH, however, significantly decreased in animals receiving either dose of L-NAME; the survival time did not differ between the L-NAME doses (Fig. 5).

In the final experimental series, there were no differences in temperature measurements (data not shown), MAP, or HR (Fig. 6) among treatment groups, either before or after EH. Additionally, the time required to induce heatstroke was not different among the three treatment groups (Fig. 7). When L-NAME was administered after EH, MAP immediately increased to levels that were not different from those attained by the respective doses given before EH (Fig. 2). The pressor response to the 100 mg/kg dose tended to be decreased relative to that attained when this dose was given during EH, although this comparison failed to reach statistical significance ($P < 0.051$). The duration of the pressor response (7 ± 1 and 8 ± 3 min for 10 and 100 mg/kg, respectively) significantly decreased relative to those observed when L-NAME was given either before or during EH. Although L-NAME increased MAP in animals in which heatstroke was induced, the survival time was not significantly altered (Fig. 7).

DISCUSSION

The major finding of this study is that acute pharmacological blockade of NO synthesis only transiently reverses the hypotension attendant to heatstroke and does not increase subsequent survival time. In fact, L-NAME administration during EH had detrimental effects on survival time, whereas pretreatment with L-NAME decreased the T_c level that induced heatstroke and tended to decrease survival time, although not significantly.

The physiological mechanism(s) underlying circulatory shock induction by EH is presently unknown. Hypotheses advanced to explain this phenomenon include endotoxemia and/or cytokine release, body fluid and electrolyte disturbances, and cellular energy de-
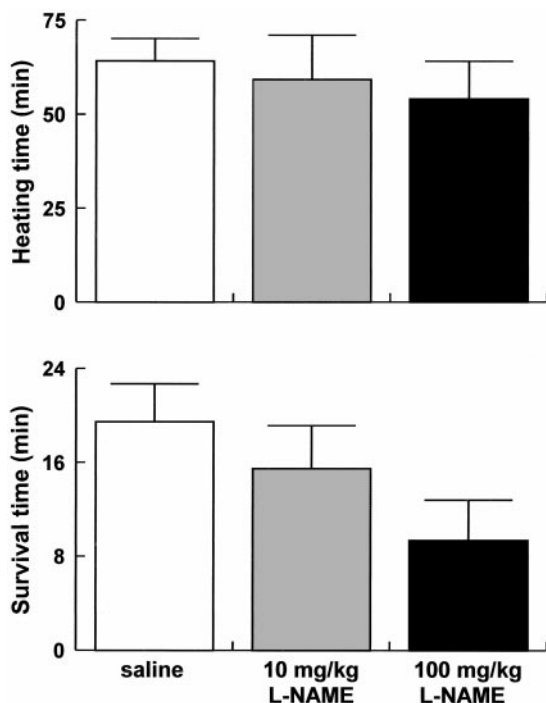
Table 2. *Temperature measurements before EH and at the end of EH in rats treated with L-NAME 30 min after the initiation of EH*

	T_c	T_{tym}	T_{sl}	T_{sr}	T_t
Before EH					
Saline vehicle	37.2 ± 0.3	36.7 ± 0.2	35.3 ± 0.4	35.8 ± 0.4	27.5 ± 1.1
L-NAME (10 mg/kg)	37.0 ± 0.0	36.6 ± 0.1	35.3 ± 0.3	35.3 ± 0.4	27.4 ± 0.4
L-NAME (100 mg/kg)	37.0 ± 0.1	37.1 ± 0.4	35.1 ± 0.3	34.6 ± 0.5	27.0 ± 0.8
End of EH					
Saline vehicle	41.9 ± 0.2	41.6 ± 0.1	40.6 ± 0.3	40.7 ± 0.2	40.1 ± 0.1
L-NAME (10 mg/kg)	41.8 ± 0.2	41.3 ± 0.3	40.3 ± 0.4	40.6 ± 0.5	40.0 ± 0.2
L-NAME (100 mg/kg)	42.2 ± 0.2	41.5 ± 0.3	40.4 ± 0.2	40.7 ± 0.5	40.1 ± 0.1

Values are means $\pm$ SE in °C.

pletion (17). What is clear, however, is that the mesenteric vasodilation that precedes EH-induced circulatory shock is not due to either decreases in sympathetic nerve activity or circulating catecholamines (15) or to a direct effect on the vascular contractile machinery (24). Although the sympathetic nervous system remains activated throughout EH-induced heatstroke, the possibility exists that vascular smooth muscle may be
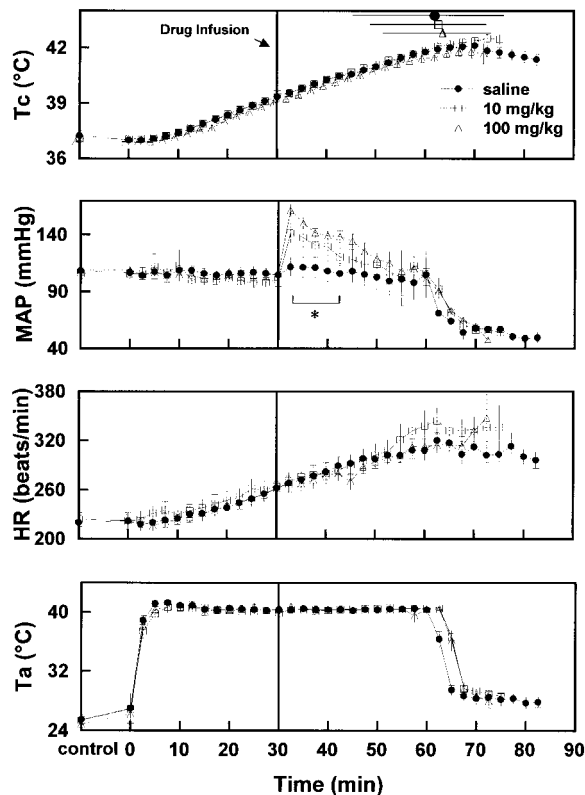

Fig. 4. Thermal and cardiovascular responses to EH. Either vehicle (saline) or L-NAME (10 or 100 mg/kg) was administered 30 min after EH was begun. Values are means $\pm$ SE. Solid horizontal lines in *top* show the range of times at which EH was discontinued for animals within that group, with the symbol placed at the group mean. Data points are shown only for times with 4 or more animals remaining in the group. Statistics were performed only through the values for 42.5 min because of missing data values thereafter. *Values for both L-NAME groups significantly different from value for saline group ($P < 0.05$).

desensitized to the effects of the catecholamine vasoconstrictors at elevated temperatures (24). Indeed, severe hyperthermia disrupts adrenoceptor function in vivo (24, 29, 50), but in vitro studies of arterial segments subjected to hyperthermia show either no effect of heat (28, 35) or an increased (24, 32) vascular responsiveness to norepinephrine and other vasoconstrictor agents (including angiotensin II). If vascular reactivity to vasoconstrictor agents is indeed reduced with hyperthermia, such a change in vascular responsiveness could contribute to the circulatory collapse associated with high T_c.

Another possible contributor to the cardiovascular events associated with heatstroke is an increased production of NO. Increased levels of NO have been associated with a number of models of circulatory shock, including endotoxemia, hemorrhage, anaphylaxis, and sepsis (31, 45, 49). Hall et al. (19) suggested that NO might contribute to the mesenteric vasodilation produced by sustained EH in rats, as NO levels in portal venous blood (but not arterial blood) increase as T_c rises >39°C. Very recently, plasma nitrite/nitrate levels (indicative of NO levels) have been demonstrated to be markedly elevated in humans with heatstroke (2). To our knowledge, the mechanism by which such elevations in NO might occur during heatstroke is presently unknown. Among the physiological mechanisms that are known to produce the release of NO is shear stress, which could certainly contribute to NO production during the hyperdynamic state induced by heat stress (13). Additionally, the production of NO has clearly been shown to be necessary for thermoregulatory vasodilation during EH in a variety of species (12, 22, 25, 41, 42, 48, 54), which might significantly contribute to elevations in NO. It is also possible that NO levels might be increased by the endotoxic response to severe heat stress, because increases in cytokines are known to increase NO production (31, 49). In this regard, recent reports suggest that *1*) hyperthermia (to a T_c of 41.5°C) produces hypoxic stress in the intestine and increases in intestinal permeability that might allow bacterial translocation from the gut (18, 27); and *2*) elevation of T_c to 41.5°C (followed by passive cooling) induces intestinal expression of the inducible isoform of NO synthase (iNOS) measured 2 and 12 h after the
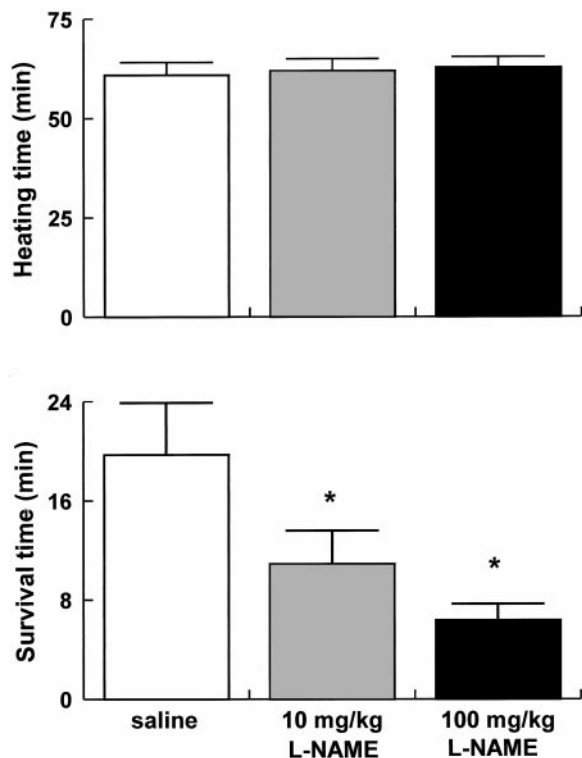
Fig. 5. EH exposure time required to reach the arbitrary end point (i.e., MAP decreased to 75 mmHg; *top*) and the subsequent survival time (*bottom*). L-NAME was administered 30 min after the start of EH. Values are means ± SE. *Significantly different from the value for control (saline) group ($P < 0.05$).

cessation of EH (20). It is therefore possible that NO synthesis might be elevated by such a series of events in clinical heatstroke or in experimental EH scenarios in which animals are allowed to survive. In experiments such as the present one in which animals are not allowed to recover from EH, however, any increases in NO synthesis produced by possible cytokine induction cannot be attributable to iNOS activation, because of the short duration of the experiment relative to the time required for iNOS and essential cofactors to be synthesized after induction (30). The possibility remains that NO synthesis might be increased if hyperthermia produces endotoxemia in these experiments, because rapid increases in NO synthesis from the constitutive isoform of NO synthase have been shown to occur in endotoxic shock (46).

Whatever the mechanism underlying the previous observations of increased NO levels in heatstroke (2, 19), pharmacological blockade of NO synthesis in vivo, however, clearly did not alter the development of hypotension during sustained EH in the present study. Preliminary results from a separate laboratory also confirm that inhibition of NO synthesis does not prevent the circulatory collapse of heatstroke (43).

In vitro investigations into the contributions of NO to vasodilation during severe heat stress have also yielded conflicting results. For example, Ryan and Gisolfi (35) demonstrated that hyperthermia of 42°C does not alter the vasodilatory responsiveness of rat

mesenteric arteries precontracted with norepinephrine to exogenous acetylcholine; because one mechanism by which acetylcholine acts to vasodilate arteries is through the release of NO by the endothelium, this result suggests that neither the release of NO nor the vascular sensitivity to NO was altered by elevation of temperature to 42°C. Interestingly, exposure of these arteries to 43°C tended to decrease relaxation to acetylcholine, although this effect did not reach statistical significance (35). In contrast, hyperthermia (41°C) decreased sensitivity to acetylcholine in preconstricted (with phenylephrine) vascular ring segments from rat mesenteric arteries, suggesting a decreased release of NO (28). Furthermore, the vasodilatory response to sodium nitroprusside was also attenuated by 41°C in this latter study, indicating that the sensitivity to NO was depressed (28). This finding of reduced vascular responsiveness to NO donors in rat mesenteric arteries at 40°C has recently been confirmed (16). Finally, the vasodilator sensitivity of precontracted (with endothelin-1) rabbit femoral arteries to acetylcholine decreased after exposure to either 41 or 44°C, suggesting that hyperthermia may inhibit production of NO; at the same time, however, the sensitivity of these arteries to sodium nitroprusside increased, implying that, despite a decreased production of NO, hyperthermia
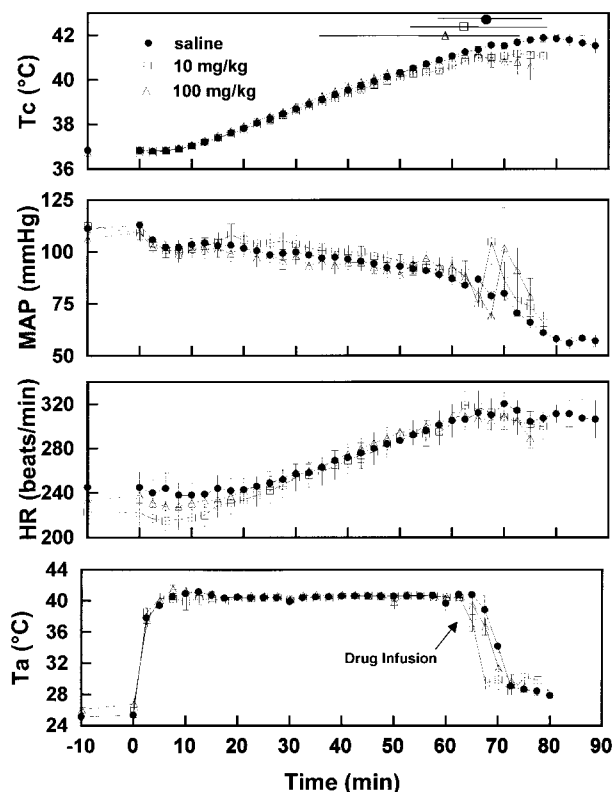

Fig. 6. Thermal and cardiovascular responses to EH. Either vehicle (saline) or L-NAME (10 or 100 mg/kg) was administered immediately after EH. Values are means ± SE. Solid horizontal lines in *top* show the range of times at which EH was discontinued for animals within that group, with the symbol placed at the group mean. Data points are shown only for times with 4 or more animals remaining in the group.
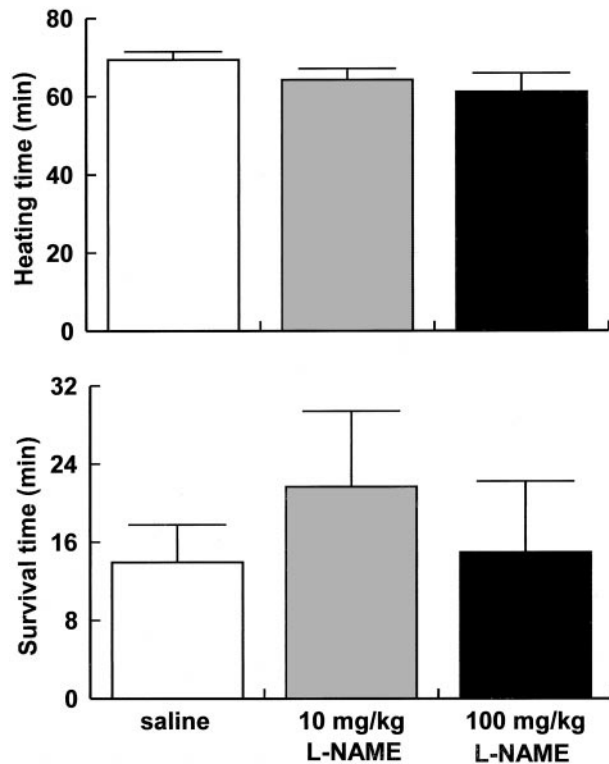
Fig. 7. EH exposure time required to reach the arbitrary end point (i.e., MAP decreased to 75 mmHg) and the subsequent survival time. L-NAME was administered on attainment of MAP end point. Values are means ± SE.

might increase the sensitivity of the vascular smooth muscle to whatever NO is present (32). It should be noted that comparison among these studies is hindered by the different experimental conditions (e.g., species, vascular beds, vasoconstrictors, and temperatures) under which each study was performed. In any event, any decrease in sensitivity to NO cannot be attributed to an altered function of a membrane receptor (as is the case with the vasoconstrictor agents discussed previously), as NO does not act as a membrane receptor (45). It would be of interest to determine whether heat stress alters the ability of NO to bind with guanylate cyclase or subsequently alters the activation of this enzyme, as previously suggested (28).

In regard to vascular reactivity issues, it was interesting to note in this study that L-NAME administration, either during or after EH, did not attenuate the resulting pressor response. In fact, the higher dose (100 mg/kg) of L-NAME given during EH actually accentuated the pressor response. To our knowledge, this is the first in vivo study in which vascular reactivity to NO synthesis inhibition after EH has been reported. This was a somewhat unexpected finding, in that our laboratory's previous work in a different hyperthermia-based model of circulatory shock induction (localized skin heating by 35-GHz microwave heating) demonstrated a dramatically reduced pressor response to L-NAME administration (36). In that animal model, localized skin heating by microwave exposure resulted

in cardiovascular events that are similar to those produced by sustained EH, but these occur at much lower T_c and higher localized skin temperature levels (14). Because pressor responses to NO synthesis inhibition were diminished, we speculated that NO levels might actually be reduced in the microwave-heating model of circulatory shock induction (36). In the present study, however, the amplitude of pressor responses to L-NAME administration did not decrease during or after EH, suggesting that there was no diminution of NO during heat stress. Interestingly, the duration of the pressor response dramatically decreased when L-NAME was administered during or after EH; to our knowledge, data such as these have not been reported previously. It is unclear why the pressor duration might have been reduced, although it is tempting to speculate that this could be a consequence of the hyperadrenergic state that occurs during EH (15), as there are well-known interactions between NO and the sympathetic nervous system (52). What is clear is that L-NAME administration in both animal models of hyperthermia (i.e., EH and microwave heating) did not increase either survival time or the time required to reach the MAP end point (36); in fact, L-NAME administration during or before hyperthermia actually decreased survival time in both heatstroke (this study) and microwave-induced circulatory shock (37). Despite the disparity in pressor responsiveness to L-NAME among these models, NO synthesis inhibition was detrimental in terms of survival benefit in both models.

Interestingly, a few studies have also reported detrimental effects of NO synthesis inhibition on survival in endotoxic and septic shock models (reviewed in Refs. 5 and 6). Although NO synthesis inhibition certainly increases systemic vascular resistance, it also decreases cardiac index and oxygen delivery in endotoxic dogs (7, 8). This decrease in cardiac index does not appear to be due to a direct depression in myocardial performance, as left ventricular stroke work index was not affected. Instead, it appears to be due to an increased afterload, as left ventricular ejection fraction decreased after NO synthesis inhibition. In these canine endotoxemia studies, NO synthesis inhibition was associated with increased mortality, especially at high doses (7, 8), just as in the present study investigating heatstroke. In this regard, NO synthase inhibition also produces a decrease in stroke volume and cardiac index in healthy human subjects and in septic patients (3, 44). Furthermore, inhibition of NO synthesis did not improve the hemodynamic response to hemorrhagic shock in swine; in fact, mortality was greater with L-NAME administration than in controls, albeit not significantly (4). NO inhibition has, therefore, been shown to decrease cardiac output and have detrimental effects in a number of species with circulatory shock induced by different etiologies. It is therefore possible that NO synthesis inhibition in the present study also increased afterload, resulting in a decrease in cardiac output. In fact, because a compensatory increase in cardiac output is a protective response to EH in the rat as in other species (47), this hypothesis becomes even

more probable; by attenuating required compensatory increases in cardiac output, NO synthesis inhibition may exacerbate detrimental effects observed in unstressed animals.

It should be emphasized that our conclusion that NO does not mediate the hypotensive state induced by sustained EH does not imply that NO plays no role in hemodynamic alterations during EH. Clearly, NO acts as a vasodilator in specific vascular beds during the early stages of EH. As examples, a NO-dependent mechanism contributes to the thermoregulatory vasodilation observed in the rat tail (54) and hindlimb (25), rabbit ear (12, 48), and human skin (22, 41, 42). In these studies, the contribution of NO was investigated at maximum core temperatures well below those sufficient to produce heatstroke; these studies, therefore, do not provide information as to whether NO contributes to the hypotension that develops at higher temperatures. Our study, although clearly demonstrating that high levels of NO do not mediate the hypotension associated with heatstroke, conversely provides no information as to the contribution of NO to hemodynamic alterations in specific locations during heat stress, as regional blood flows were not measured. Although our study was not designed to determine whether NO plays a role in thermoregulatory vasodilation, an anecdotal observation was that NO synthesis inhibition did not significantly alter the rate of heating.

In this study, NO synthesis inhibition was accomplished using L-NAME, which is one of the most commonly used inhibitors in studies investigating contributions of NO to the development of shock (31, 45, 49). Because L-NAME was administered as a pretreatment in one set of experiments, a quite valid question is whether NO synthesis inhibition was maintained throughout the period of EH. On administration, L-NAME is rapidly hydrolyzed to form N^G-nitro-L-arginine, which is thought to be the actual inhibitor (33, 40). Although the in vivo half-life of L-NAME itself has been calculated to be only $\sim$7.5 min in anesthetized rabbits, plasma levels of N^G-nitro-L-arginine quickly rise and remain elevated without diminution for at least 30 min (40). Recently, Conner et al. (9) demonstrated that the vasoactivity of L-NAME (as measured by vasoconstriction in a number of organs) persists for at least 3 h after a single intraperitoneal bolus injection of L-NAME in conscious rats. We therefore believe that NO synthesis inhibition was adequately maintained throughout the period of EH.

Interestingly, the application of EH to the ketamine-anesthetized rat model did not produce the pressor responses previously noted in conscious, chloralose-anesthetized (26) or urethane-anesthetized (unpublished observations) rats. Despite a lower baseline HR than that in conscious rats, a pronounced tachycardia was induced by EH, as has been noted in rats maintained with other anesthetic regimens (26; unpublished observations). It should be noted that administration of L-NAME did not produce reflex bradycardia; our laboratory has previously observed this lack of

bradycardic response to L-NAME administration in rats anesthetized with either pentobarbital sodium or ketamine (37). It is therefore probable that the use of ketamine as the anesthetic agent somewhat altered the hemodynamic responses to EH and blunted the reflex responses to the increase in blood pressure elicited by L-NAME. It has been reported, however, that ketamine preserves cardiovascular function better than other anesthetics during compromised situations such as experimental shock (39). Taking these caveats into consideration, we emphasize that our results apply only to the ketamine-anesthetized rat model and should be confirmed in either conscious models or those using other anesthetics.

In conclusion, acute pharmacological inhibition of NO synthesis, either before or during EH, did not protect the animal from subsequent heatstroke. Indeed, NO synthesis inhibition at these time points had detrimental effects on survival time and the temperature at which heatstroke was reached. L-NAME administration after the induction of heatstroke also failed to ameliorate the hypotensive state and was not of survival benefit. Taken together, these results suggest that the vasodilator action of NO does not mediate the development of the severe hypotension that accompanies heatstroke in ketamine-anesthetized rats.

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